Neutrophil trafficking to lymphoid tissues: Physiological and pathological implications

Running title: Neutrophils in lymphoid tissues in health and diseases

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Abstract

Recent advances have provided evidence for the involvement of neutrophils in both innate and adaptive immunity, robustly challenging the old dogma that neutrophils are short-lived prototypical innate immune cells solely involved in acute responses to microbes and exerting collateral tissue damage. There is now ample evidence showing that neutrophils can migrate into different compartments of the lymphoid system where they contribute to the orchestration of the activation and/or suppression of lymphocyte effector functions in homeostasis and during chronic inflammation, such as autoimmune disorders and cancer. In support of this notion, neutrophils can generate a wide range of cytokines and other mediators capable of regulating the survival, proliferation and functions of both T and B cells. In addition, neutrophils can directly engage with lymphocytes and promote antigen presentation. Furthermore, there is emerging evidence of the existence of distinct and diverse neutrophil phenotypes with immunomodulatory functions that characterise different pathological conditions, including chronic and autoimmune inflammatory conditions. The aim of this review is to discuss the mechanisms implicated in neutrophil trafficking into the lymphoid system and to provide an overview of the immuno-regulatory functions of neutrophils in health and disease in the context of adaptive immunity.

Key words: Neutrophils, lymphatic system, adaptive immunity, auto-immunity, chronic inflammation, Cancer

Introduction

Neutrophils are short-lived immune cells that are commonly accepted as being the first leukocyte sub-type to be recruited in large numbers from the blood circulation into inflamed tissues. Their migration through the cellular and matrix components of blood vessel walls is a tightly regulated process involving intricate cellular and molecular interactions, as summarised in recent articles [1-6]. This phenomenon is fundamental for an effective innate immune response against infections or acute injury, providing a host defence mechanism that clears tissues of invading pathogens, dead cells and potentially harmful debris. Neutrophil diapedesis also plays an important role in regulating the phenotype and activation state of emigrated neutrophils at sites of inflammation [7]. Key effector functions of neutrophils include phagocytosis and killing of intracellular or extracellular pathogens, tissue remodelling and secretion of chemotactic and immunomodulatory molecules that can further regulate the recruitment and activation of other pro-inflammatory leukocytes [8-11]. To facilitate these functions, activated neutrophils can also release a wide range of granular proteases and cytotoxic factors, as well as generate a host of reactive oxygen species. Whilst such capabilities are essential armoury for destroying microorganisms, they can also cause collateral damage to host tissues, rendering clearance of apoptotic neutrophils an essential component of resolving an inflammatory response [12,13]. Another form of cell death program for neutrophils is via NETosis, a dynamic process associated with the generation of neutrophil extracellular traps (NETs) [14-16]. NETs are generated through the release of decondensed chromatin and granular enzymes into the extracellular space surrounding leukocytes as means of controlling the dissemination of infectious microorganisms [14,17,18]. Whilst the role of NETs in the direct killing of pathogens remains contentious [14,19,20], there is strong evidence to indicate that the formation of NETs *in vivo* can be detrimental to the host. Pathological induction of NETs, such as that induced under conditions of sterile injury (e.g. ischemia-reperfusion injury) can also cause tissue damage and indeed NETosis has been implicated to the pathogenesis of a wide range of non-infectious inflammatory disorders [21-

24]. Collectively, excessive recruitment, activation and/or inefficient clearance of infiltrated neutrophils is now categorically associated with the development of numerous acute pathological conditions such as myocardial infarction, stroke and tissue damage caused by ischemic insults [22,25,26] and there is now a growing interest in the pathogenic potential of neutrophils in chronic conditions such as cancer [27-29]. Furthermore, the association of neutrophils with multiple autoimmune disorders (e.g. rheumatoid arthritis, lupus, multiple sclerosis, Crohn's disease and vasculitis) [21,30-34] has invigorated the interest in neutrophils as potential players in regulation of adaptive immunity.

Nearly 50 years ago, whilst studying the trafficking of immune cells in sheep, Smith and colleagues discovered that the neutrophil "endgame" was not limited to apoptosis within inflamed tissues but that these cells could be detected in the peripheral lymph of the animals [35]. The authors speculated that this response provided a means for neutrophils to recirculate back into the blood, as opposed to contributing to adaptive immune responses taking place in the draining lymph nodes (LNs). This hypothesis was upheld for a long time due to the difficulty in culturing neutrophils for prolonged periods *in vitro* and the general and well-accepted assumption that the neutrophil life expectancy in the blood circulation did not exceed one day. However, advancements in techniques for tracking neutrophils *in vivo*, and the overall better understanding of neutrophil biology, have unequivocally demonstrated that neutrophils can exhibit prolonged survival both *in vitro* and *in vivo* [36]. For example, whilst human neutrophils are reported to exhibit a blood circulation period of up to 5 days [37], cytokines such as GM-CSF [38,39], bacteria-derived products [40], hypoxic conditions [41] and diapedesis through blood endothelium [42,43] can protect neutrophils from rapid cell death. Such findings have led to greater acceptance of neutrophils as regulators of the adaptive immunity as supported by a large body of evidence demonstrating that neutrophils can migrate into secondary lymphoid organs such as lymph nodes (LNs) during bacterial and parasitic infections as well as during vaccination challenge protocols [44-47]. These works also reported on the ability of neutrophils to secrete numerous immunomodulatory molecules affecting lymphocytes [48-50]

as well as to directly interact with lymphocytes acting as antigen presenting cells (APCs) [51-56]. Collectively there is now a renewed interest in models related to neutrophil trafficking into the lymphoid system and the pathophysiological consequences of this response, concepts that are reviewed below.

Neutrophil trafficking to lymphoid tissues

Neutrophil migration to lymphoid organs was first demonstrated in animal models through localising neutrophils within the draining LNs of tissues infected with microorganisms or following vaccine challenge [44-47,57]. The development and use of advanced imaging tools, such as intravital confocal microscopy, in conjunction with genetically modified animals exhibiting fluorescently-tagged neutrophils (e.g. *LysM-GFP-ki* mice), enabled detailed analysis of the dynamics of neutrophil-lymphatic vessel interactions as well as the role of specific molecular cues involved in this process (Table 1). These studies provided direct evidence for neutrophils migrating to LNs via afferent lymphatics present in inflamed tissues. Interestingly, this trafficking response was rapid (within 6-12hrs post insult) and transient as very few neutrophils could be detected in the LNs past 48hrs [46,57]. The first molecular pathway linked with this response involved CCR7 and its cognate ligands CCL21 and CCL19 [45]. Importantly, the work of Beauvillain and colleagues demonstrated the presence of intracellular stores (possibly secretory vesicles) of CCR7 in both human and murine neutrophils isolated from the blood and bone marrow, respectively. Interestingly, whilst CCR7 was almost un-detectable on the cell surface of neutrophils, the introduction of a purification step to enrich the neutrophil population *in vitro* enabled the detection of the molecule on the membrane. These findings suggested that priming of leukocytes was essential for the trafficking of CCR7 from intracellular stores to the cell membrane. Indeed, stimulation of human neutrophils with the cytokine GM-CSF could promote their migration towards a CCL21/CCL19 chemotactic gradient *in vitro*, a response that was potentiated by LPS or IL17. *In vivo*, we and others have demonstrated that upon immunisation with complete Freund's adjuvant (CFA), CCR7 deficient

mice have reduced numbers of neutrophils migrating into tissue-associated lymphatic capillaries and into draining LNs, as compared to wild-type control littermates [45,57]. Our study also provided evidence that immunisation of the animals with CFA induced the local release of endogenous TNF, a response essential for the control of neutrophil entry into lymphatic capillaries in a CCR7-dependent manner. Furthermore, the trafficking of CCR7-deficient neutrophils through afferent lymphatic vessels was completely suppressed in TNF-induced inflammation [57]. Interestingly, significant CCR7 expression was detected on the cell surface of tissue-infiltrated neutrophils but not on cells from the blood circulation, or on tissue-infiltrated neutrophils deficient in TNF receptors (both TNFRI and TNFRII). Collectively, these findings provide compelling evidence to indicate the necessity of priming for neutrophil migration into the lymphoid system and identify tissue-derived TNF as a key modulator of *in vivo* expression and function of CCR7 on neutrophils. Other studies have suggested that the CXCR4/CXCL12 axis is critical for neutrophil entry into the lymphatic system [53,58]. CXCR4 is a chemokine receptor expressed at low levels on the surface of mature healthy neutrophils; but this molecule is upregulated on the membrane of aged neutrophils, a response associated with the egress of senescent neutrophils from the circulation [59-61]. In a study using a murine model of *S. aureus* infection, a specific inhibitor of CXCR4, AMD3100, was shown to significantly reduce the migration of neutrophils into afferent lymphatic vessels and draining LNs, whilst CCR7-deficient neutrophils exhibited normal trafficking to the lymphatic system [53]. Similar results were obtained in a mouse model of immunisation associated with pre-activation of neutrophils with immune complexes [58]. Differential involvement of distinct chemokine axes in regulating neutrophil entry into the lymphatics might depend on the inflammatory models used, the degree of activation of neutrophils or the potential existence of yet not described tissue-specific mechanisms. Another chemokine implicated in human neutrophil migration into the lymphatic system is the prototypical neutrophil chemoattractant CXCL8. A study by Rigby and colleagues recently demonstrated that human dermal lymphatic endothelial cells (LECs) can secrete this chemokine and promote the migration of human neutrophils through LEC monolayers *in vitro* [62]. Similarly, isolated LECs from murine skin

exhibited enhanced gene expression of CXCL1 (a homologue of human CXCL8) upon stimulation [63]. However, blockade of CXCL1 protein, or its receptor CXCR2, had no effect on murine neutrophil recruitment to lymphatic vessels *in vivo* [53,57], highlighting potential discrepancies between putative *in vivo* and *in vitro* – and species - scenarios. In addition to chemokines, adhesion molecules such as ICAM-1 and VCAM-1 have been reported to be expressed by stimulated LECs and to support human and mouse neutrophil-lymphatic vessel interactions via binding to leukocyte β2 integrins (e.g. Mac-1) both *in vitro* and *in vivo* [53,57,62]. For instance, Mac-1/ICAM-1 interaction is critical for the attachment and crawling of murine neutrophils along the luminal aspect of lymphatic endothelium *in vivo* [57]. Similarly, Mac-1 blockade inhibited the entry of neutrophils into lymphatics of mouse skin that had been locally injected with bacteria [53] whilst another neutrophil-expressed integrin, LFA-1, is apparently dispensable for neutrophil intravasation into lymphatic vessels [58].

Several studies have also demonstrated the capacity of blood circulating neutrophils to enter lymph nodes via high endothelial venules (HEVs) during infection, post immune complex activation and antigen sensitisation [53,58,64]. To date the only chemotactic axis described to be important for the migration of neutrophils through HEVs is CXCR4/CXCL12 axis, whilst a role for CCR7 and its cognate ligands CCL21/CCL19 have been completely ruled out [53,58]. Other molecules associated with neutrophil-HEV interactions are P- & L-selectins and their cognate ligand PSGL-1 as well as endothelial cell ICAM-1 and leukocyte integrins Mac-1 and LFA-1 [53,58].

Collectively, there is now ample evidence to demonstrate the capacity of neutrophils to migrate during inflammation into LNs via two distinct routes, though the molecular pathways of such events require further exploration. Nevertheless, the fact that LN neutrophils can originate from either the blood circulation or inflamed tissues suggests potential differential modes of neutrophil-mediated regulation of the adaptive immunity. The following section discusses the immuno-modulatory functions of neutrophils in the context of lymphocyte activation.

Neutrophil regulation of lymphocyte functions

Recent advances in neutrophil biology, including studies detailed in the previous section, have acknowledged these cells as key players at the interface of innate and adaptive immunity in both physiological homeostasis and pathological inflammation. The rapid and transient nature of their trafficking to LNs, with a dwelling time in these organs not exceeding 2-7 days following an initial inflammatory insult [53,64], has led to the hypothesis that neutrophils can facilitate the transport of pathogens and antigens into LNs. It is considered that the latter are subsequently transferred to resident DCs and macrophages already present in secondary lymphoid organs through engulfment of neutrophil-derived vesicles (e.g. apobodies, exosomes or micro-vesicles). Furthermore, it is envisaged that through this cascade of events macrophages and conventional APCs such as DCs, can process and present exogenous antigens to lymphocytes [46,47]. This concept is supported by the detection of apoptotic neutrophils within LNs of *S.aureus* infected animals [53]. In addition to this notion, it is now clear that activated neutrophils can secrete numerous immune modulatory molecules that can directly stimulate the recruitment, activation and functions of lymphocytes [48-50]. More importantly, neutrophils can impact the regulatory functions of lymphocytes via direct neutrophil-lymphocyte interactions and antigen presentation [65]. For example, several studies have demonstrated the existence of the so-called neutrophil-DC hybrids, an activated neutrophil sub-type that exhibit characteristics of DCs (e.g. expressing MHC-II CD80 & CD86) and capable of presenting exogenous antigen to both CD4+ and CD8+ T lymphocytes *in vivo* and *in vitro* [51,66-68]. This phenomenon was confirmed by confocal imaging that showed the dynamics of cell contacts between neutrophils and lymphocytes in mouse models of infection and immunisation [46]. In humans, neutrophils can also stimulate the antigen-specific proliferation of both naïve and memory T cells through MHC-II expression [69]. Furthermore, neutrophils can positively and directly modulate B cell activation, survival and differentiation by mean of secretion of cytokines such as B-cell-Activating Factor of the tumor necrosis factor Family (BAFF) [70] and A Proliferation-Inducing Ligand (APRIL) [71]. Whilst neutrophils are

not usually seen in the germinal centres of LNs, a subpopulation has been found in the peri-follicular areas and marginal zone of the spleen in both humans and mice [72,73]. These cells, termed B-helper neutrophils, have been reported to induce T-cell independent production of IgG and IgA (following immunoglobulin class switching) via the production of large amount of BAFF, APRIL, CD40L and interleukin-21. B helper neutrophils may therefore represent a central mechanism for enhancing antibody production and effective humoral responses in a T-cell independent manner [74-77]. Direct Interactions between neutrophils and B cells have also been observed in real time in lymph nodes of *S.aureus* infected animals [64].

In recent years, the homogeneity of neutrophil population has become increasingly questionable, with detection of neutrophils exhibiting distinct functions and profiles leading to the concept of different subsets [78-81]. Several studies have indeed demonstrated that alternatively activated and/or HEV-recruited neutrophils can regulate the adaptive immune response by inhibiting B and T lymphocyte responses, in particular in the context of antibody production during vaccination challenge and infections [46,53,64,82,83]. Whilst the mechanisms associated with such functions are not fully understood, neutrophil suppressive properties on T lymphocyte activities involve the release of reactive oxygen species, nitric oxide or Arginase 1 in close vicinity of targeted lymphocytes [84,85]. Neutrophils can also directly inhibit T cell functions through cell-cell interactions in a Mac-1 or PD-L1 dependent manner during HIV infections, systemic endotoxemia and cancer [86-89]. Furthermore, whilst neutrophil depletion can increase the production of antigen-specific IgG and IgM, during *S.aureus* infection, B-helper neutrophils were shown to limit the production of IgM through release of TGF-β1 [64]. Finally, recent studies have also investigated the role of specific subtypes of neutrophils in the context of regulatory T cell (Treg) expansion and recruitment. For instance, an elegant study by Nadkarni and colleagues has demonstrated that neutrophils sensitised to pregnancy hormones promote the differentiation of a unique population of FOXP3+ CD4+ Tregs with a specific secretory phenotype (release of IL-10, VEGF and IL-17)

via the transfer of neutrophil-derived proteins such as forkhead box protein-1 to naive T cells [90].

Taken together, there is now unquestionable evidence supporting neutrophil trafficking into the lymphatic system and the ability of these cells to regulate lymphocyte functions. These works have promoted more interest into the potential role of neutrophils in chronic disorders, including autoimmune diseases, as discussed below.

Diversity of neutrophil phenotype and pathogenic potential in chronic and autoimmune diseases

The notable and now widely accepted diversity of neutrophil functions has placed a spotlight on the potential existence of distinct neutrophil-subsets and their association with a broad range of pathologies (see examples in Figure 1). This includes subtypes of neutrophils that can exert stimulatory and suppressive effects on lymphocyte functions and the potential association of these cells to chronic disorders such as cancer, and autoimmune diseases, such as systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA) [80,91,92]. One example relates to granulocytic myeloid-derived suppressor cells (PMN-MDSCs), detected in cancer patients, considered by some researchers as a subset of neutrophils [80,85,93,94]. This section reviews recent findings associated with neutrophil trafficking to lymphoid tissues in cancer and chronic inflammatory and autoimmune pathologies.

The duality of neutrophil functions in cancer

In human cancer, tumour-associated neutrophils have been classified as having anti-tumour (N1) and pro-tumour (N2) properties [91,95]. There is also a body of evidence supporting the presence of increased numbers of PMN-MDSCs in blood, within the tumour microenvironment and peripheral lymphoid organs (tumour-draining LNs and spleen) in this pathology in humans

and mice [96]. The dominant view is that neutrophils and PMN-MDSCs are important contributors to tumour progression through their ability to promote angiogenesis, proliferation and metastasis of cancer cells [93,94,97], as supported by experimental murine models of cancer [98]. Furthermore, N2 neutrophils and PMN-MDSCs have been shown to exhibit immunosuppressive properties via PD-L1-dependent immunosuppression of Th1 cell proliferation [88] and to promote the expansion and recruitment of regulatory T lymphocytes via the secretion of CCL17 [99-101]. Of note, a distinct sub-population of human neutrophils (exhibiting neutrophil-DC hybrid characteristics) has been discovered within the tumour-microenvironment and draining LNs during the early stage of lung cancer [102,103]. These tumour-associated neutrophils were reported to induce efficient anti-tumour responses from memory CD8+ and CD4+ T cells *in vitro* and expressed CCR7 at their surface [104]. In support of these findings, a recent study has described the presence of $CXCR2^{+}$ neutrophils exhibiting IL-6 secretory pro-inflammatory phenotype in the draining LN of gastric tumours [105]. At present, the literature lacks further detailed information regarding the route, mechanisms of migration and activity of such distinct populations of neutrophils during the development of the pathology *in vivo.* Yet, in the context of anti-tumour therapy, using a mouse model of colon carcinoma, Brackett and colleagues demonstrated that photodynamic therapy (PDT) was also associated with the rapid recruitment of neutrophils (i.e. within 4hrs post PDT) into tumour-draining LNs through HEVs in an IL-17 and CXCR2/CXCL2 (but not CXCL1)-dependent manner [106,107].The authors also showed the importance of L-selectin and peripheral node addressin (PNAd) in neutrophil entry through HEVs in this model. Functionally, PDT has been associated with the development of neutrophil-dependent and tumour-specific primary and memory CD8(+) T cell responses through the direct induction of T lymphocyte proliferation and/or survival [108].

Neutrophils in chronic inflammatory and auto-immune disorders

Neutrophils with immuno-modulatory activities have also been detected in ageing, now regarded by some researchers as a low grade chronic inflammatory state (i.e. inflammageing). Accordingly, in aged mice neutrophils have been detected in high numbers in both T-cell and B-cell areas of the secondary lymphoid organs (i.e. LNs and spleen) [109]. The increased neutrophil trafficking to lymphoid organs was associated with enhanced life-span of the infiltrated neutrophils and their altered phenotype. Specifically, in healthy aged mice, lymphoid tissue neutrophils expressed an activated phenotype characterised by high levels of Mac-1 and ICAM-1 and the concomitant synthesis of both pro-inflammatory and anti-inflammatory cytokines TNF and TGFβ, respectively. At present it is unclear what the role of these neutrophils is, but it is speculated that the atypical phenotype of lymphoid organ neutrophils could contribute to ageing-associated dysregulation of normal adaptive immune responses, e.g. in infections [109].

In autoimmune pathologies such as rheumatoid arthritis (RA), human synovial neutrophils express transcripts and proteins of MHC-II and co-stimulatory molecules CD80 and CD86 during the early phase of the disease [110,111]. *In vitro*, freshly isolated synovial neutrophils are capable of stimulating a proliferative response in T-helper cells [111]. Whilst rather intriguing, such seminal studies did not demonstrate where (e.g. tissue localization) and when (e.g. phase of the disorder) during RA such neutrophil responses were functionally relevant to disease progression. Indeed, it would be important to elucidate if neutrophils can regulate T cell functions in RA *in vivo* within the secondary lymphoid organs. Related to this caveat, using a murine model of lupus, Bird and colleagues demonstrated that neutrophils can preferentially form close interactions with T cells in the early phase of the disease, whilst in the advanced stage, they mainly accumulate in B cell areas of the spleen, reminiscent of a B-helper phenotype [112]. Furthermore, transcriptome analysis demonstrated that neutrophils exhibited high expression of PD-L1, TGFβ and IL-1RA during the early disease phase. The immunosuppressive activity of neutrophils during this period was confirmed by the negative effect of neutrophil depletion on disease progression, germinal centre formation and

production of anti-double strand DNA antibodies. Conversely, during the late phase, neutrophils enriched in splenic B-cell regions increased their expression of TNF and BAFF, indicating that they may contribute to the expansion of auto-reactive B lymphocytes during disease progression [112]. Interestingly, B-helper neutrophils have been shown to be more susceptible to NETosis [73] and hence it has been suggested that, due to their localization and specific phenotypic characteristics, these cells may promote the expansion and survival of auto-reactive B cells. A role for B-helper neutrophils in supporting adaptive autoimmune responses has indeed been reported in many autoimmune disorders, including, RA, SLE and in anti-neutrophil cytoplasmic antibody-associated (ANCA) vasculitis [72]. The mechanism of action of these neutrophils is not completely understood but their enhanced capacity to generate NETs was suggested to provide an abundant source of auto-antigens characteristic of these pathologies [17,113-115]. Supporting this concept, a recent study by Gestermann and colleagues demonstrated that NET components could directly activate memory B cells through TLR9 stimulation, leading to the production of pathogenic autoantibodies in a T cell-independent manner [116]. Moreover, NETs can also stimulate plasmacytoid dendritic cells to secrete interferon-α which in turn, promotes NETosis of neutrophils. This cascade perpetuates a vicious cycle that can be observed in pathologies such as vasculitis, SLE, psoriasis, and type-1 diabetes [117-120].

The number of PMN-MDSCs have also been reported to increase in RA and SLE patients [121-123] as well as in several experimental animal models of lupus [124], collagen-induced arthritis [125], experimental autoimmune encephalomyelitis (EAE) [126], type-1 diabetes and inflammatory bowel disease [127,128]. Whilst many studies have correlated MDSCs with disease severity, their role in regulating lymphocyte responses remains controversial [129]. Indeed, whilst in RA, SLE and EAE, PMN-MDSCs have been shown to inhibit both proliferation of T helper-cells and their production of cytokines (e.g. interferon-γ and IL-2) in an arginase-1-dependent manner, these cells also produce pro-inflammatory cytokines (e.g. IL-1 and TNF). The latter can promote the differentiation of naïve T lymphocytes into Th17 cells,

suggesting that in the disease inflammatory context, PMN-MDSCs can exhibit a pathogenic phenotype [122,130,131].

Overall, whilst the current literature supports the concept of neutrophils orchestrating a pathogenic response during autoimmune and chronic inflammatory disorders, further investigations are required to determine if and how neutrophil trafficking into lymphoid organs might be contributing to the pathoetiology of these diseases.

Conclusion and perspectives

In addition to their essential role in protecting the host against acute harmful insults, recent studies have identified a broader role for neutrophils in both physiological and pathological immunity, and hence the development of numerous chronic and autoimmune inflammatory disorders. Specifically, it is now clear that, despite being short-lived as compared to other immune cells, neutrophils can traffic into lymphoid tissues and contribute to shaping key adaptive immune responses. As such, current evidence supports the notion that in addition to dendritic cells and macrophages, neutrophils can act as a cellular bridge between innate and adaptive immunity in both health and disease. Whilst our understanding of the mechanisms of neutrophil trafficking and functions into lymphoid tissues are yet to be fully investigated, it is now accepted that depending on the nature, duration or the site of the insult, neutrophils can enter the lymphatic system by using both afferent lymphatic vessels as well as venular portals within LNs. Once in secondary lymphoid organs, neutrophils can act as immune-modulatory cells through cytokine production and via direct cell-cell interaction with other immune cells. Accordingly, neutrophils can orchestrate elaborate cellular and humoral responses as well as exert regulatory effects on lymphocyte functions. The development of new technologies applied to investigate the dynamics of neutrophil behaviour, phenotype and trafficking in both patient samples *ex vivo* and in animal models of inflammation *in vivo* has led to the identification of different subtypes of neutrophils with distinct regulatory functions in health and

disease. It is still debated if neutrophil subsets might represent an acquired phenotype, and/or level of activation through environmental molecular cues, or are in fact ontogenically separate cell populations. Irrespective of this, renewed interest in this phenotypic diversity and associated varied effector functions, in conjunction with better understanding of the spatiotemporal localisation of neutrophils is opening challenging research opportunities. In particular such works could pave the way towards addressing outstanding questions regarding the diverse functions of neutrophils during the development of autoimmune pathologies. Most importantly, association of different neutrophil subtypes with the pathogenesis of defined disorders has the potential for stratifying patients in terms of disease severity as well as identify new therapeutic targets in chronic and autoimmune conditions and cancer.

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Author contributions statement

MBV performed the literature search and wrote the manuscript. SN contributed to the writing and revision of the manuscript. Both authors approved the final version.

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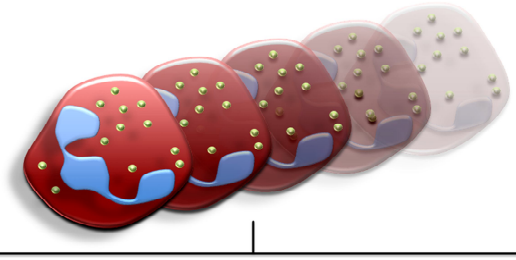
Figure 1: Neutrophils and the regulation of the adaptive immune response in diseases

Neutrophils have been implicated in the regulation of T and B cell activities in many pathological scenarios including inflammageing, cancer, and autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus. Neutrophils can both stimulate and immunosuppress lymphocyte functions through various mechanisms due to the plasticity of their phenotype, localisation and environmental priming. For instance they can secrete activating cytokines or act as antigen presenting cells to stimulate lymphocyte proliferation and auto-antibody production (e.g. via NETosis). In contrast, other subtypes of neutrophils have been shown to directly inhibit T cell activation through the release of TGFβ, nitric oxide (NO), arginase-1 (Arg-1) as well as through the expression and direct engagement of PD-L1 with T cells.

Table 1: Molecules implicated to neutrophil migration into the lymphoid system.

Molecule	Surface expression	Role in entry via lymphatic capillaries	Role in entry via high endothelial venules	Inflammatory model (when relevant)	References
CCR7	• Low neutrophil surface expression necessitating priming: → *in vitro* (GM-CSF/IL-17/LPS [a] or TNF [b]) → *in vivo* (post-extravasation, TNF-dependent [b]) • Expressed on tumour-associated neutrophil N1 type [a]	Yes but depends on the nature of the reaction	No	• *In vitro* stimulation [a] • Immunisation (CFA) [b] • TNF-induced inflammation [b] • Early lung tumour [a]	[45], [53], [57], [107]
CXCR4	• Low surface level on mature neutrophils • High surface level on aged neutrophils	Yes, (but depends on the nature of the reaction	Yes	• *S.aureus* infection [b] • Immunisation with immuno-complex activation [b] • Immunisation (CFA) [b]	[53], [57], [58]
CXCR2	High levels on mature naïve neutrophils	Yes, *in vitro* No, *in vivo*	*in vivo* during PDT	• *In vitro* stimulation of dermal LECs [a] • *S.aureus* infection [b] • Immunisation (CFA) [b] • Photodynamic Therapy in cancer (PDT) [b]	[53], [57], [62], [107]
CD54 (ICAM-1)	Up-regulation on LVs and LECs upon inflammation (TNF dependent)	Yes, (promote adhesion and luminal crawling)	Yes	• *S.aureus* infection [b] • Immunisation, TNF-induced inflammation [b] • Immunisation with immuno-complex activation [b] • *In vitro* stimulation of dermal LECs [a]	[53], [57], [58], [62]
CD11b (Mac-1)	High expression on all neutrophils	Yes, (promote adhesion and luminal crawling)	Yes	• *S.aureus* infection [b] • Immunisation, TNF-induced inflammation [b] • Immunisation with immuno-complex activation [b] • *In vitro* stimulation of dermal LECs [a]	[53], [57], [58], [62]
CD11a (LFA1)	Expressed on all neutrophils	Rarely	Yes	• *S.aureus* infection [b] • Immunisation with immuno-complex activation [b]	[53], [58]
CD62L (L-selectin)	Expressed on neutrophils, shed upon stimulation/extravasation	No	Yes	• *S.aureus* infection [b] • Immunisation with immuno-complex activation [b] • Photodynamic Therapy in cancer [b]	[53], [58], [107]
CD62P (P-selectin)	Endothelial selectin, upregulated upon stimulation of ECs	Not Tested	Yes	• Immunisation with immuno-complex activation [b]	[58]
CD62E (E-selectin)	Endothelial selectin, upregulated upon stimulation of ECs	*in vitro* only	Yes	• *In vitro* stimulation of dermal LECs [a]	[58]
CD168 (PSGL-1)	Selectin ligand expressed by both LECs and neutrophils	No	Yes	• *S.aureus* infection [b] • Immunisation with immuno-complex activation [b]	[53], [58]
PNAd (Peripheral Node Addressin)	Expressed on HEVs	No	Yes	• *S.aureus* infection [b] • Immunisation with immuno-complex activation [b]	[53], [58]

[a] shown in humans
[b] shown in mouse models



	Inflammageing	Cancer	Autoimmune disorder	
			Systemic lupus erythematosus	*Rheumatoid arthritis*
Pro-inflammatory	✓ **Localisation:** . Spleen (T & B cell zones) . LNs ✓ **Phenotype:** . Mac-1^{high}, CD54^{high} . Increased lifespan ✓ **Function:** . TNF release (Spleen only)	✓ **Localisation:** . Tumour . Blood ✓ **Phenotype:** . N2 type & PMN-MDSCs . iNOS^{+}, Arg-1^{high}, PD-L1^{+} ✓ **Function:** . Inhibit anti-tumour T cell response . Recruit T Reg (CCL17)	✓ **Localisation:** . Spleen (B cell zone), blood ✓ **Phenotype:** . "classical" neutrophils . PMN-MDSCs ✓ **Function:** . Secrete TNF, BAFF . Forms NETs . Stimulate pDCs (IFNα) . Activate B cells (NETs & TLR9) . Polarise Th17 cells (PMN-MDSC)	✓ **Localisation:** . Synovial fluid ✓ **Phenotype:** . Neutrophil/DC hybrid (MHC-II^{+}, CD80^{+}, CD86^{+}) . PMN-MDSCs (Arg1^{+}, IL-1^{+}) ✓ **Function:** . Activate CD4^{+} T cell (*in vitro*) . NETosis & auto-antibody production . Polarise Th17 cells (PMN-MDSC)
Immuno-suppressive	✓ **Localisation:** . Spleen (T & B cell zones) . LNs ✓ **Phenotype:** . Mac-1^{high} & CD54^{high} . Increased lifespan ✓ **Function:** . TGFβ production	✓ **Localisation:** . Early tumour . Tumour-draining LN (through HEVs) ✓ **Phenotype:** . N1 type . CCR7^{+}, MHC-II^{+}, CD86^{+}, CD54^{+} ✓ **Function:** . Activate CD4^{+} & CD8^{+} T cells	✓ **Localisation:** . Spleen (T cell zone) ✓ **Phenotype:** . "classical" neutrophils . PMN-MDSCs, PD-L1^{+} ✓ **Function:** . Secrete TGFβ & IL-1RA . Inhibit CD4^{+} T cells	✓ **Localisation:** . Blood, Spleen ✓ **Phenotype:** . PMN-MDSC ✓ **Function:** . Inhibit CD4^{+} T cell (*in vitro*)